From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 13 July 2001 (13.07.01)	
International application No. PCT/EP00/09671	**Applicant's or agent's file reference** 0050/051748
International filing date (day/month/year) 02 October 2000 (02.10.00)	**Priority date** (day/month/year) 06 October 1999 (06.10.99)
Applicant AMBERG, Wilhelm et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

17 April 2001 (17.04.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Elisabeth KÖNIG

Telephone No.: (41-22) 338.83.38

4150066

Form PCT/IB/331 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
0050/051748		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/09671	02/10/2000	06/10/1999
Applicant		
BASF AKTIENGESELLSCHAFT		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: all in part

Present claims 1-10 relate to "endothelin blocker" and alphavbeta3 integrin receptor antagonists, both of these groups comprising an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. In addition, it must also be noted that the prior art does not necessarily define compounds according to their agonistic or antagonistic activities, but rather in structural terms, rendering a complete search according to such functional definitions impossible.
Consequently, the search has been carried out for the general concepts of endothelin blocker and alphavbeta3 receptor antagonists.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
T/EP 00/09671

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K45/06 A61P9/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MORRIS J ET AL: "SMALL MOLECULE APPROACHES TO THE PREVENTION OF RESTENOSIS" CURRENT PHARMACEUTICAL DESIGN, BENTHAM SCIENCE PUBLISHERS, SCHIPHOL, NL, vol. 1, no. 4, 1995, pages 469-482, XP001022941 ISSN: 1381-6128 abstract page 470, column 1, paragraph 2 -page 473, column 1, paragraph 1 page 475, column 2, paragraph 2 -page 477, column 1, paragraph 1 page 481, column 1, last paragraph --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 January 2002	07/02/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Didelon, F

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	SANJAY SRIVATSA S ET AL: "SELECTIVE ALPHA V BETA 3 INTEGRIN BLOCKADE POTENTLY LIMITS NEOINTIMAL HYPERPLASIA AND LUMEN STENOSIS FOLLOWING DEEP CORONARY ARTERIAL STENT INJURY: EVIDENCE FOR THE FUNCTIONAL IMPORTANCE OF INTEGRIN ALPHA V BETA 3 AND OSTEOPONTIN EXPRESSION DURING NEOINTIMA FORMATION" CARDIOVASCULAR RESEARCH, vol. 36, no. 3, December 1997 (1997-12), pages 408-428, XP001020872 ISSN: 0008-6363 abstract figures 7-9	1-10
Y	WO 99 11626 A (SMITHKLINE BEECHAM CORP ;HEERDING DIRK A (US); SAMANEN JAMES M (US) 11 March 1999 (1999-03-11) page 8, line 13 -page 9, line 2 page 9, line 26 - line 29 claims 1,18,19	1-10
Y	WO 98 08840 A (HOFFMAN WILLIAM F ;HUTCHINSON JOHN H (US); MEISSNER ROBERT S (US);) 5 March 1998 (1998-03-05) page 3, line 1 - line 3 page 200, line 18 - line 26 claims 1,8,9	1-10
Y	WO 99 31061 A (HUTCHINSON JOHN H ;MEISSNER ROBERT S (US); ASKEW BEN C (US); DUGGA) 24 June 1999 (1999-06-24) page 56, line 19 - line 25 page 190, line 16 - line 25 claims 1,25-27	1-10
Y	WO 98 27070 A (HERGENROEDER STEFAN ;BASF AG (DE); JANSEN ROLF (DE); KLING ANDREAS) 25 June 1998 (1998-06-25) page 40, line 1 - line 14 page 1, line 41 - line 42; claims 1,6	1-10
Y	DE 198 09 144 A (BASF AG) 9 September 1999 (1999-09-09) claims 1,4,6	1-10
Y	KIRCHENGAST M & MÜNTER K: "Endothelin and restenosis" CARDIOVASCULAR RESEARCH, vol. 39, 1998, pages 550-555, XP002092668 ISSN: 0008-6363 page 552, column 2, paragraph 2 -page 554, column 2	1-10

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: all in part

Present claims 1-10 relate to "endothelin blocker" and alphavbeta3 integrin receptor antagonists, both of these groups comprising an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. In addition, it must also be noted that the prior art does not necessarily define compounds according to their agonistic or antagonistic activities, but rather in structural terms, rendering a complete search according to such functional definitions impossible.
Consequently, the search has been carried out for the general concepts of endothelin blocker and alphavbeta3 receptor antagonists.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/09671

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9911626	A	11-03-1999	EP	1027337 A1	16-08-2000
			JP	2001514253 T	11-09-2001
			WO	9911626 A1	11-03-1999
WO 9808840	A	05-03-1998	AU	724191 B2	14-09-2000
			AU	4086597 A	19-03-1998
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			US	6211184 B1	03-04-2001
WO 9931061	A	24-06-1999	AU	739811 B2	18-10-2001
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			EP	1040098 A1	04-10-2000
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			NO	20003114 A	16-08-2000
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			WO	9931061 A1	24-06-1999
			US	6048861 A	11-04-2000
			US	6297249 B1	02-10-2001
WO 9827070	A	25-06-1998	DE	19652763 A1	25-06-1998
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			HR	970686 A1	31-10-1998
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			WO	9944998 A1	10-09-1999
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			HR	20000650 A1	30-06-2001
			NO	20004351 A	01-09-2000
			PL	342806 A1	02-07-2001
			SK	11752000 A3	10-05-2001
			TR	200002545 T2	21-11-2000
			ZA	9901738 A	11-10-2000

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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199 62 998.6	24 December 1999 (24.12.1999)	DE
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100 39 998.3	11 August 2000 (11.08.2000)	DE

(71) Applicant *(for all designated States except US)*: **BASF AKTIENGESELLSCHAFT** [DE/DE]; 67056 Ludwigshafen (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **AMBERG, Wilhelm** [DE/DE]; Schälzigweg 79, 68723 Schwetzingen (DE). **KLING, Andreas** [DE/DE]; Riegeler Weg 14, 68239 Mannheim (DE). **HORNBERGER, Wilfried** [DE/DE]; Goldener Winkel 14, 67434 Neustadt (DE).

(74) Common Representative: **BASF AKTIENGESELLSCHAFT**; 67056 Ludwigshafen (DE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/24827 A2

(54) Title: INHIBITORS OF THE ENDOTHELIN SIGNALLING PATHWAY AND $\alpha_v\beta_3$ INTEGRIN RECEPTOR ANTAGONISTS FOR COMBINATION THERAPY

(57) Abstract: The invention relates to the use of an endothelin blocker in combination with an $\alpha_v\beta_3$ integrin receptor antagonist for the treatment or prevention f diseases, particularly to the use of a pharmaceutical composition, comprising an endothelin blocker and an $\alpha_v\beta_3$ integrin receptor antagonist, for the treatment or prevention of cardiovascular disorders, particularly for the treatment or prevention of restenosis after vessel injury or revascularisation treatment and to the pharmaceutical composition itself.

ME1

Inhibitors of the Endothelin Signalling Pathway and $\alpha_v\beta_3$ Integrin receptor antagonists for Combination Therapy

The invention relates to the use of an endothelin blocker in combination with an $\alpha_v\beta_3$ integrin receptor antagonist for the treatment or prevention of diseases, particularly to the use of a pharmaceutical composition, comprising an endothelin blocker and an $\alpha_v\beta_3$ integrin receptor antagonist, for the treatment or prevention of cardiovascular disorders, particularly for the treatment or prevention of restenosis after vessel injury or revascularisation treatment and to the pharmaceutical composition itself.

Percutaneous transluminal coronary angioplasty (PTCA) was first introduced into the therapy of patients with coronary artery stenosis in the late seventies. In the two decades since this method has become the standard therapy for patients suffering from all forms of coronary artery disease. The success rate of the procedure itself has increased from 61 percent in the late seventies to well over 90 percent from the mid-eighties onwards. However, long-term success of PTCA remains limited by late restenosis caused by vessel wall proliferation that occurs in 20 to 40 percent of all patients to such an extent that a second PTCA is necessary [Anderson VH, Smalling RW, Serruys PW. Mechanical devices. In: Willerson JT, Cohn JN (eds). Cardiovascular Medicine. New York, Edinburgh, London: Churchill Livingstone, 1995:617-651].

As in the mid-90s about 500,000 primary PTCA procedures were carried out in the USA and about 200,000 in Europe, this accounts for more than 230,000 patients per year eligible for a second invasive procedure due to recurrent angina. A hypothetical reduction of the incidence of restenosis by 10-15 percent per year would reduce annual treatment costs by almost 1.5 billion dollars in the USA and Europe alone.

Up to now a large number of clinical trials performed to investigate whether systemic administration of drugs which were effective in animal models of restenosis were efficacious in men have failed. The reason for this discrepancy between pre-clinical and clinical studies could be that doses effective in experimental settings have not been applicable to patients due to other cardiovascular effects of th se drugs [Pratt RE, Dzau VE. Pharmacological strategies to prevent restenosis. Circulation 1996;93:848-852].

Meanwhile revascularisation by balloon dilatation, stentimplantation, laser or rotablator are used not only in coronary arteries, but in all other large arteries accessible if atherosclerotic lessions make such an intervention necessary (Stenosis of renal arteries, carotid arteries, femoral and brachial arteries). Thus the number of patients having problems with restenosis after any such intervention steadily increases posing a huge therapeutical and economic (reduction in health care costs) potential for drugs effectively inhibiting restenosis.

Endothelin (ET), a 21 amino acid peptide, has been described as the most potent endogenous vasoconstrictor known. [Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988; 332:411-415]. Its precursor is big-Endothelin, which is cleaved to ET by ECE (endothelin converting enzyme). ET binds in an autocrine/paracrine fashion to two different specific high affinity receptors, named ET_A and ET_B. Within the vasculature ET_A receptors are only located on smooth muscle cells (SMCs) leading to vasoconstriction and SMC proliferation. In addition, a variable portion of ET_B receptors were also described on SMCs promoting at that location the same effect as ET_A receptors via the same intracellular signalling pathways [Rubanyi GM, Polokoff MA. Endothelins: Molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. Pharmacol Rev 1994;46:325-415].

It is known, that ET_A receptor antagonists effect on restenosis. The selective ET_A receptor antagonist A 127722 was tested in pigs with coronary artery stents [McKenna CJ, Burke SE, Opgenorth TJ, et al., Selective ET_A receptor antagonism reduces neointimal hyperplasia in a porcine coronary stent model, Circulation 1998, 97, 2551-2556]. After 28 days of oral treatment (b.i.d.) a maximal reduction in neointima formation of about 30% has been reported.

Arterial injury after revascularisation also triggers the expression of vascular membrane-bound protein heterodimers called integrins especially of integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ [Corjay MH, Diamond SM, Schlingmann KL, Gibbs SK, Stoltenborg JK, Racanelli AL. AlphaVbeta3, alphaVbeta5, and osteopontin are coordinately upregulated at early time points in a rabbit model of neointima formation. J Cell Biochem 1999;75:492-504] and it is known since 1994 that formation of neointimal hyperplasia as the leading cause of restenosis can be inhibited by antagonists to integrin $\alpha_v\beta_3$ [Choi ET, Engel L, Callow AD, Sun S, Trachtenberg J, Santor S, Ryan US. Inhibition of neointimal hyperplasia by blocking $\alpha_v\beta_3$ integrin

with a small peptide antagonist GpenGRGDSPCA J Vasc Surg 1994;19:125-134].

First experimental hints came from the work of Sriramarao and co-workers which could show that endothelial attachment and spreading as basic mechanisms of angiogenesis is mediated by integrins $\alpha_2\beta_1$ and $\alpha_v\beta_3$ and could be inhibited by the integrin $\alpha_v\beta_3$ specific antibody LM-609 and by RGD-containing peptides [Sriramarao P, Mendler M, Bourdon M Endothelial cell attachment and spreading on human tenascin is mediated by alpha 2 beta 1 and alpha v beta 3 integrins. J Cell Science 1993;105:1001-1012].
Based on the clinical success of the anti-platelet integrin $\alpha_{IIb}\beta_3$ antibody abciximab (c7E3 Fab, Reopro®) which led to sustained suppression of ischemic complications (endpoints death , MI or repeat intervention) after coronary interventions [Topol EJ, Ferguson JJ, Weisman HF, Tcheng JE, Ellis SG, Kleiman NS, Ivanhoe RJ, Wang AL, Miller DP, Anderson KM, Califf RM. Long-term protection from myocardial ischemic events in a randomized trial of brief integrin beta3 blockade percutaneous coronary intervention. EPIC Investigator Group. Evaluation of Platelet IIb/IIIa Inhibition for Prevention of Ischemic Complication. JAMA 1997;278:479-84] and the finding that it has similar affinity to both integrin $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ [Tam SH, Sassoli PM, Jordan RE, Nakada MT. Abciximab (ReoPro, chimeric 7E3 Fab) demonstrates equivalent affinity and functional blockade of glycoprotein IIb/IIIa and alpha(v)beta3 integrins. Circulation 1998;98:1085-1091] it was suspected that besides acute thrombosis abciximab was also able to prevent vascular restenosis.
This anti-proliferative effect could be demonstrated in a porcine coronary injury model, where a selective integrin $\alpha_v\beta_3$ blockade by the peptidic compound XJ 735 potently reduced neointimal hyperplasia by 43% and led to 2.9 fold less lumen stenosis [Srivatsa SS, Fitzpatrick LA, Tsao PW, Reilly TM, Holmes DR Jr, Schwartz RS, Mousa SA. Selective alpha v beta 3 integrin blockade potently limits neointimal hyperplasia and lumen stenosis following coronary arterial stent injury: evidence for the functional importance of integrin alpha v beta 3 and osteopontin expression during neointima formation. Cardiovasc Res. 1997;36:408-28].

In addition integrin $\alpha_v\beta_3$ antagonists have been shown to themselves exhibit some antithrombotic activity due to inhibition of $\alpha_v\beta_3$-mediated platelet function like adhesion to the vessel wall [Gawaz M, Neumann FJ, Dickfeld T, Reininger A, Adelsberger H, Gebhardt A, Schomig A. Vitronectin receptor (alpha(v)beta3) mediates platelet adhesion to the luminal aspect of endothelial

cells: implications for reperfusion in acute myocardial infarction. Circulation. 1997;96:1809-18].

Both receptors, namely ET receptors and integrin $\alpha_v\beta_3$, play a role in restenosis after vascular injury both in animal models and in man. By inhibiting either of the two principles, a 30 to 40 percent reduction of neointima formation could be achieved in the relevant experimental models.

It is an object of the present invention to provide an effective method of treatment or prevention of cardiovascular disorders, particularly of restenosis after revascularisation, with acceptable side effects and advantageous properties.

We have found that this object is achieved by using an endothelin blocker in combination with an $\alpha_v\beta_3$ integrin receptor antagonist.

By combining compounds which act as ET blockers and $\alpha_v\beta_3$ integrin receptor antagonist either in one formulation or as a kit-of-parts combination by applying both separately via the same or different routes, it is possible to achieve a reduction of restenosis significantly more pronounced than one of the two treatments alone at the given doses. The combination of an ET blocker and an $\alpha_v\beta_3$ integrin receptor antagonist in doses too low to be effective alone is as least as effective as a high mono-therapy with either agent and has less potential for side-effects than one principle alone.

Therefore, the invention relates to the use of an endothelin blocker in combination with an $\alpha_v\beta_3$ integrin receptor antagonist for the manufacture of medicaments for the treatment or prevention of diseases, particularly of cardiovascular disorders, particularly of restenosis after vessel injury or revascularisation treatment.

Cardiovascular disorders are, for example, atherosclerosis, restenosis after vessel injury or revascularisation treatment, angioplasty (neointima formation, smooth muscle cell migration and proliferation), myokard infarkt or heart failure.

In a preferred embodiment, the combination according to the invention can be used for the manufacture of medicaments for the treatment or prevention of restenosis after vessel injury or revascularisation treatment.

According to the invention, restenosis preffered means the sum of angiographic end points and clinical events, i.e. late (30 days after intervention and later) proliferative thickening of the wall and loss of minimal lumen diameter (MLD) of the vessel subjected to angioplasty together with the clinical endpoints death, myocardial infarction and repeat intervention (PTCA).

Restenosis can occure after vessel injury or revascularisation treatment. Revascularisation treatment preferred means methods of percutaneous transluminal angioplasty (PTA), such as balloon dilatation, stentimplantation, laser or rotablator. These methods are used not only in coronary arteries (percutaneous transluminal coronary angioplasty (PTCA)), but also in other large arteries accessible if atherosclerotic lessions make such an intervention necessary (Stenosis of renal arteries, carotid arteries, femoral and brachial arteries).

According to the invention, endothelin blocker means an inhibtor of the Endothelin Signalling Pathway such as, for example, endothelin receptor antagonists (ET antagonist), ECE inhibitors, antibodies against ET or ECE or modulators of expression of ET-precoursor proteins or ET-receptors, particularly inhibitors of the Big-ET expression.

Preferred endothelin blockers are ET antagonists, ECE inhibitors, or Antibodies against ET or ECE, most preferred endothelin blockers are ET antagonist.

Preferred ECE Inhibitors within the scope of the invention are compounds which have a K_i value of 1µM or less. Most preferred are those ECE Inhibitors which have a K_i value of 100nM or less and mostly preferred are those ECE Inhibitors which have a K_i value of 10nM or less.

Suitable for the combination therapy of the invention are in principle all ECE inhibitors, for example peptidic and non-peptidic inhibitors, preferred are non-peptidic inhibitors, more preferred such which are orally available, such as

Pohosphoramidon;
CGS-31447: 1-{[(1S)-2-[1,1'-biphenyl]-4-yl-1-(1H-tetraazol-5-yl)ethyl]amino}-3-(1-naphthyl)propylphosphonic acid;
CGS-34043: {[(1S)-2-dibenzo[b,d]furan-3-yl-1-(1H-tetraazol-5-yl)ethyl]amino}methylphosphonic acid;
CGS-35066: (2S)-3-dibenzo[b,d]furan-3-yl-2-[(phosphonomethyl)amino] propanoic acid; CGS-35339: (2S)-3-dibenzo[b,d]furan-3-yl-2-{[(diphenoxyphosphoryl)methyl] amino}propanoic acid;

CGS-35066; WS-79089A: 1,6,9,14-tetrahydroxy-3-(2-hydroxypropyl)-7-methoxy-8,13-dioxo-5,6,8,13-tetrahydrobenzo[a]naphthacene-2-carboxylic acid; WS-75624A: (6-[2-(6-hydroxyheptyl)-1,3-thiazol-4-yl]-4,5-dim thoxy-2-pyridinecarboxylic acid); PD-069185: N-(1-ethyl-3-piperidinyl)-6-iodo-2-(trichloromethyl)-4-quinazolinamine; SCH-54470: N-(1-(Hydoxy(1(R)-(N-alpha-(methylsulfonyl)-L-lysylamino)-2-phenylethyl)phosphinylmethyl)cyclopentylcarbonyl)-L-tryptophan dilithium salt; RU-69296: (2S)-2-{[2-(3-bromobenzyl)-3-sulfanylpropanoyl]amino}-3-(1H-indol-3-yl)propanoic acid; RU-69739: (2S)-2-{[2-(4-bromobenzyl)-3-sulfanylpropanoyl]amino}-3-(1H-indol-3-yl)propanoic acid; KC-12792-2-AB; SLV-306: ((3S,2'R)-3-(1-(2'-(Ethoxycarbonyl)-4'-phenyl-butyl-)-cyclopentan-1-carbonylamino)-2,3,4,5-tetrahydro-2-oxo-1H-benzapin-1-acetic acid; FR-901533;

Preferred Endothelin receptor antagonists within the scope of the invention are substances which have a K_i value of 1µM or less for either the ET_A receptor or the ET_B receptor or for both receptors at the same time. Most Preferred are those endothelin receptor antagonists which have a K_i value of 100nM or less and mostly preferred are those endothelin receptor antagonists which have a K_i value of 10nM or less. The K_i value of endothelin receptor antagonists can be measured as described in DE 19636046 A1.

Suitable endothelin receptor antagonists for the combination therapy of the invention are in principle all endothelin receptor antagonists, peptidic and non-peptidic antagonists, for example as described in WO 96/22978, WO 98/27070, WO 98/09953, EP 617001, WO 98/22482, WO 97/30045, WO 9963936, WO 9833780, WO 9854116, WO9842709, WO 9841521, WO 9849162, WO 9717342, WO 9813366, WO 9739000, WO 9730045, WO 2000001389, WO 9937639, WO 9912916, WO 05132, WO 9728154, WO 9612706, WO 9827091, DE 19612101, DE 19609597, US 5716984, US 5939446, US 5922681, US 6048893 or GB 2337048, preferred are non-peptidic antagonists, more preferred such which are orally available.

Examples for peptidic endothelin receptor antagonists are:

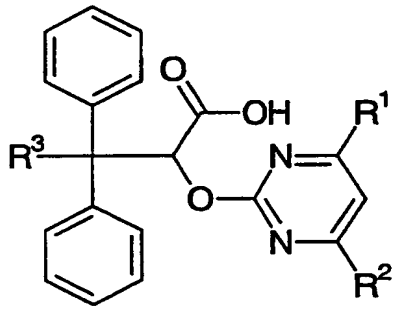
FR-139317 (Perhydroazepine-1-ylcarbonyl-L-leucyl-(1-methyl)-D-tryptophyl-[3-(2-pyridyl)]-D-alanine); FR-901367 (2-Acetamido-3-[[1,4,4a,5,6,6a,7,12,12a,12b-decahydro-4a,8,12a,12b-tetrahydroxy-3-methyl-1,7,12-trioxobenz[a]anthracene-6a-yl]thio]propionic acid); BE-18257B (Cyclo(-D-Trp-D-Glu-L-Ala-allo-D-Ile-L-Leu-)); BQ-123 (Cyclo(-D-Trp-D-Asp-L-Pro-D-Val-L-Leu-)); TAK-044 (Cyclo(-4-oxo-4-(4-phenyl-1-piperazinyl)-L-2-aminobutanoyl-L-Asp-D-2-(2-thienyl)glycyl-L-Leu-D-Trp-D-Asp-) di-sodium salt); PD-142893 (N-Acetyl-(3,3-diphenyl-D-ala-

nine)-L-Leu-L-Asp-L-Ile-L-Ile-L-Trp); PD-156252 (N-Acetyl-2-D-(10,11-dihydro-5H-dibenzo(a,d)cycloheptene-5-yl)-glycyl-L-Leu-L-Asp-L-Ile-N-methyl-L-Ile-L-Trp disodium salt); BQ-485 (Perhydroazepin-1-ylcarbonyl-L-leucyl-D-tryptophyl-D-tryptophan); Cochinmicin I or Myricerone caffeic acid ester.

Examples for non-peptidic ET receptor antagonists are:

Sitaxsentan (N-(4-Chloro-3-methylisoxazol-5-yl)-2-(2-(6-methyl-3,4-methylendioxy-1-yl)acetyl)thiophen-3-sulfonamide); TBC-3214 (N-(2-acetyl-4,6-dimethylphenyl)-3-{[(4-chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thiophenecarboxamide); TBC-3711; SB-209670 ((1S,2R,3S) 1-(3,4-methylendioxyphenyl)-3-(2-(carboxymethoxy)-4-methoxyphenyl)-5(prop-1-yloxy)indan-2-carboxylic acid); Bosentan (4-tert-Butyl-N-(6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)(2,2'-bipyrimidin)-4-yl)benzene-sulfonamide); PD-156707 (2-(3,4-Methylendioxyphenyl)-4-(4-methoxyphenyl)-4-oxo-3- (3,4,5-trimethoxybenzyl)-but-2-ene acid sodium salt); L-749329 (4-(2-(4-Isopropylphenylsulfonamido)-1-(3,4-methylenedioxyphenyl)-2-oxoethoxy)-3-propylbenzoic acid); L-754142 (4-(2-(4-Isopropylphenylsulfonamido)-1-(3,4-methylendioxyphenyl)-2-oxoethoxy)-3-propylbenzoic acid dipotassium salt); Enrasentan ((1S,2R,3S) 1-(3,4-Methylendioxyphenyl)-3-(2-(2-hydroxyethoxy)-4-methoxyphenyl)-5(prop-1-yloxy)indan-2-carboxylic acid); A-127722 (trans-trans-2-(4-methoxyphenyl)-4-(3,4-methylendioxyphenyl)-1-(2-(N,N-dibutylamino)-2-oxoethyl)-pyrrolidine-3-carboxylic acid); Abtrasentan (ABT-627 ([2S-(2a,3b,4a)]-2-(4-methoxyphenyl)-4-(3,4-methylendioxyphenyl)-1-(2-(N,N-dibutylamino)-2-oxoethyl)-pyrrolidine-3-carboxylic acid)); EMD-94246 (N-(2,1,3-Benzothiadiazol-5-yl)-5-(dimethylamino)naphthalin-1-sulfonamide potassium salt); ZD-1611 (3-(4-(3-(N-(3-Methoxy-5-methylpyrazin-2-yl)sulfamoyl)pyridin-2-yl)phenyl)-2,2-dimethylpropionic acid); K-8794 (N-(2,6-dimethylphenyl)-3-(6-(4-t-butylphenylsulfonylamino)-5-(2-methoxyphenoxy)-2-(2-pyrimidinyl)-4-pyrimidinyloxy) propionamide); A-182086 ((2a,3b,4a)-2-(3-Fluor-4-methoxyphenyl)-4-(3,4-methylendioxyphenyl)-1-(2-(pentylsulfonyl)propyl-amino)ethyl-pyrrolidine-3-carboxylic acid); PD-163070 ((2*Z*)-2-(1,3-benzodioxol-5-yl)-3-[3-(dimethylamino)benzyl]-4-(4-methoxyphenyl)-4-oxo-2-butenoic acid sodiumsalt); PD-166557 ((2*Z*)-3-(3-aminobenzyl)-2-(1,3-benzodioxol-5-yl)-4-(4-methoxyphenyl)-4-oxo-2-butenoic acid sodium salt); Ro-61-1790 (N-{6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-[2-(1H-tetraazol-5-yl)-4-pyridinyl]-4-pyrimidinyl}-5-methyl-2-pyridinesulfonamide disodium salt) BMS-193884 (N-(3,4-dimethyl-5-isoxazolyl)-4'-(1,3-oxazol-2-yl)[1,1'-biphenyl]-2-sulfonamide); BMS-207940; SB-209598 (3-[2-(carboxymethoxy)-4-methoxyphenyl]-1-[(6-chloro-1,3-benzo-

dioxol-5-yl)methyl]-1*H*-indole-2-carboxylic acid); SB-209834 ((2*E*)-2-(1,3-benzodioxol-5-ylmethyl)-3-(2-butyl-1-methyl-1*H*-imidazol-5-yl)-3-[2-(carboxymethoxy)-4-methoxyphenyl]-2-propenoic acid); A-206377 ((2*S*,3*R*,4*S*)-4-(1,3-benzodioxol-5-yl)-1-[2-(dibutylamino)-2-oxoethyl]-2-[2-(2-oxo-1-pyrrolidinyl)ethyl]-3-pyrrolidinecarboxylic acid); EMD-122801 ((2*Z*)-2-(2,1,3-benzothiadiazol-5-yl)-4-(4-methoxyphenyl)-4-oxo-3-(3,4,5-trimethoxybenzyl)-2-butenoic acid sodium salt); Tezosentan (N-{6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-[2-(1H-tetraazol-5-yl)-4-pyridinyl]-4-pyrimidinyl}-5-isopropyl-2-pyridinesulfonamide disodium salt); AC-61-0612(N-{6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-[2-(1H-tetraazol-5-yl)-4-pyridinyl]-4-pyrimidinyl}-5-isopropyl-2-pyridinesulfonamide); T-0201 (N-[6-{2-[(5-bromo-2-pyrimidinyl)oxy]ethoxy}-5-(4-methylphenyl)-4-pyrimidinyl]-4-(2-hydroxy-1,1-dimethylethyl)benzenesulfonamide sodiumsalt); J-104132 ((5*S*,6*R*,7*R*)-5-(1,3-benzodioxol-5-yl)-2-butyl-7-{2-[(2*S*)-2-carboxypropyl]-4-methoxyphenyl}-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine-6-carboxylic acid) and compounds of the general formula I:



I

wherein R^1, R^2 and R^3 are:

R^1 C_1-C_4-Alkyl, C_1-C_4-Alkoxy;

R^2 C_1-C_4-Alkyl, C_1-C_4-Alkoxy;

R^3 C_1-C_8-Alkyl which may carry a phenyl which may carry up to 2 identical or different C_1-C_4-Alkoxy radicals.

Preferred compounds of the formula I are compounds, wherein R^1, R^2 and R^3 are

R^1 C_1-C_2-Alkyl, C_1-C_2-Alkoxy;

R^2 C_1-C_2-Alkyl, C_1-C_2-Alkoxy;

R^3 C_1-C_2-Alkyl which may carry a phenyl which may carry up to 2 identical or different C_1-C_2-Alkoxy radicals.

Orally available ET-receptor antagonists are listed, for example, in Douglas, S. A., Trends in Pharmacol. Sci., 18, 408-12, 1997, preferred are ABT-627, A-182086, PD163070, PD166557, Bosentan, TBC-11252 or ZD-1611.

Most preferred ET antagonists are
(S)-2-(4,6-Dimethoxy-pyrimidin-2-yloxy)-3-methoxy-3,3-diphenyl-propionic acid,
(S)-2-(4,6-Dimethyl-pyrimidin-2-yloxy)-3-(2-(3,4-dimethoxy-phenyl)ethoxy)-3,3-diphenylpropionic acid,
(S)-2-(4,6-Dimethyl-pyrimidin-2-yloxy)-3-methoxy-3,3-diphenyl-propionic acid or
(S)-2-(4,6-Dimethyl-pyrimidin-2-yloxy)-3,3-diphenylbutanoic acid.

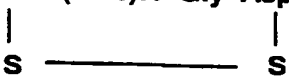
Preferred $\alpha_v\beta_3$ integrin receptor antagonists within the scope of the invention are substances which show an IC_{50} value of 100nM or less for the inhibition of vitronectin binding to integrin $\alpha_v\beta_3$ in an ELISA assay, which is, described for example in DE 19919218.9 (German application number).

Suitable $\alpha_v\beta_3$ integrin receptor antagonists for the combination therapy of the invention are, in principle, all $\alpha_v\beta_3$ integrin receptor antagonists, for example as described in Pitts et al.; J. Med. Chem. 2000, 43, 27-40; Batt et al., J. Med. Chem. 2000, 43, 41-51; Miller et al., Bioorg. Med. Chem. Lett. 9, 1999, 1807-1812; Keenan et al., Bioorg. Med. Chem. Lett. 9, 1999, 1801-1806; Rockwell et al., Bioorg. Med. Chem. Lett. 9, 1999, 937-942; Samanen et al., Current Pharm. Design 1997, 3, 545-584; Miller et al., J. Med. Chem. 2000, 43, 22-26; Hartmann and Duggan, Exp. Opin. Invest. Drugs 2000, 9 (6), 1281-1291; Miller et al., Drug Discovery Today 2000, 5 (9), 397-408; DE 19919218.9 (German application number), DE 19948269.1 (German application number), DE 19962998.6 (German application number), DE 10027514.1 (German application number), DE 10028575.9 (German application number), DE 10039998.3 (German application number), WO 9952879, WO 9835917, WO 0000486, WO 0017197, WO 0031067, WO 9843962, WO 9926945, WO 9950249, WO 9958162, WO 0000481, US 6056958, WO 43787, WO 9637492, WO 9723480, WO 9733887, WO 9748395, WO 9748444, WO 9823608, US 5,849,736, DE 19626701, EP 0796855A1, DE 19653645, DE 19653646, DE 19653647, EP 796855, EP 820988, EP 820991, EP 853084, EP 854145, US 5990145, WO 9915506, WO 9915507, WO9932457, WO 9937621, WO 9959992, EP 928790, EP 928793, US 6001855, WO 00024724, WO 9825892, WO 9965944, WO 0048603, WO 9938849, WO 9952872, DE 19534016, DE 19548709, DE 19653036, DE

19654483, DE 19705450, DE 1971300, DE 19725368, DE 19842415, DE 19850131, EP 683173, EP 710657, EP 741133, EP 771 818, WO 9714716, WO 9723451, WO 9738009, WO 9744333, WO 9800395, WO 9818764, WO 9827112, WO 9835949, WO 9901472, WO 9910371, WO 9931126, WO 0003973, WO 0026212, WO 9532710, WO 9726250, WO 9737655, WO 9808518, WO 9808840, WO 9818460, WO 9818461, WO 9831359, WO 9844797, WO 9846220, WO 9901472, WO 9930709, WO 9930713, WO 9931061, WO 9931099, WO 0006169, WO 0009503, US 5981546, US 6017925, US 6017926, WO 9967230, WO 9734865, FR 2768734-A1, FR 2768736-A1, WO 0032578, US 5639765, US 5681820, US 5852210, US 5972986, US 6013651, WO 9708145, WO 9736858, WO 9736859, WO 9736860, WO 9736861, WO 9736862, WO 9944985, WO 9944994, WO 9951638, WO 9952896, WO 0009143, WO 0038665, WO 0038715, WO 0038719, WO 0038786, WO 9600574, WO 9600730, WO 9606087, WO 9626190, WO 9701540, WO 9724119, WO 9724122, WO 9724124, WO 9724336, WO 9814192, WO 9815278, WO 9829561, WO 9830542, WO 9840488, WO 9905107, WO 9906049, WO 9911626, WO 9915170, WO 9915178, WO 9915508, WO 9945927, WO 0007544, WO 0033838 or WO 9933798, particularly, the following proteins, peptidic and nonpeptidic compounds.

Proteins and peptidic $\alpha_v\beta_3$ integrin receptor antagonists:

LM 609 (vitaxin, Pharmaprojects),
abciximab (c7E3 Fab, Reopro®,Pharmaprojects),
Peptides and peptidomimetics of Arg-Gly-Asp and derivatives thereof like:
cyclo(RGDfV), As-Pen-RGDC-OH, cyclo[RGD-Mamb-P], XJ 735 (cyclo[R-G-D-Mamb-A]), XK 002 (cyclo[(NMe)R-G-D-(2-amino-1,3-thiazol-4yl-acetic acid)-V]), DMP 728 (cyclo[[(NMe)R-G-D-Mamb-DAbu]), SK+F 107260



EMD 121974 (cyclo[R-G-D-f-(NMe)V]) and any other RGD containing peptides.

Non-peptidic $\alpha_v\beta_3$ integrin receptor antagonists:
(2*R*)-2-[((2*R*)-2-{3-[(3-{[amino(imino)methyl]amino}propanoyl)amino]phenyl}-3-carboxy propanoyl)amino]-3-methylbutanoic acid, 3-[8-(2-{[amino(imino)methyl]amino}ethyl)-1-benzyl-2-oxo-1,2,3,5-tetrahydro-4*H*-1,4-benzodiazepin-4-yl]propanoic acid, 2,3-dihydroxypropyl 2-{[(benzyloxy)carbonyl]amino}-4-({9,10-dimethoxy-4-[(*E*)-2-(1,4,5,6-tetrahydropyrimidin-2-yl)hydrazono]-1,2,3,3a,4,5,6,10b-octahydrobenzo[ ]azulen-8-yl}oxy)butanoate, (2*S*)-2-{[(benzyloxy)carbo-

nyl]amino}-3-[({(4*S*)-4-[3-(4,5-dihydro-1*H*-imidazol-2-ylamino)propyl]-2,5-dioxoimidazolidin-1-yl}acetyl)amino]propanoic acid, L-7418415 ((2*S*)-2-[(phenylsulfonyl)amino]-3-({4-[2-(1,4,5,6-tetrahydropyrimidin-2-ylamino)ethoxy]benzoyl}amino)propanoic acid), (2*S*)-2-{[(4-isobutylphenyl)sulfonyl]amino}-3-[({5-[3-(pyridin-2-ylamino)propyl]-4,5-dihydroisoxazol-3-yl}carbonyl)amino]propanoic acid, (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[({4-[4-(4,5-dihydro-1*H*-imidazol-2-ylamino)butanoyl]piperazin-1-yl}carbonyl)amino]propanoic acid, (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[({4-[4-(4,5-dihydro-1*H*-imidazol-2-ylamino)propanoyl]piperazin-1-yl}carbonyl)amino]propanoic acid, SD-186 ((2*S*)-2-[(phenylsulfonyl)amino]-3-[{(8-(pyridin-2-ylamino)methyl}-1-oxa-2-aza-spiro[4.5]dec-2-en-3-yl]carbonyl)amino]propionic acid), SD-183 ((2S)-2-[(phenylsulfonyl)amino]-3-[({8-[(pyridin-2-ylamino)methyl]-1-oxa-2-azaspiro[4.5]dec-2-en-3-yl}carbonyl)-amino]propanoic acid, SD-983 ((2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[({3-[3-(4,5-dihydro-1*H*-imidazol-2-ylamino)propoxy]isoxazol-5-yl}carbonyl)amino]propanoic acid), XT-199 ((2*S*)-3-[({3-[3-(4,5-dihydro-1*H*-imidazol-2-ylamino)propoxy]isoxazol-5-yl}carbonyl)amino]-2-[(phenylsulfonyl)amino]propanoic acid), SG-545 (Methyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[({3-[3-(4,5-dihydro-1*H*-imidazol-2-ylamino)propoxy]isoxazol-5-yl}carbonyl)amino]propanoic acid), SM 256 ((2*S*)-3-[({1-[3-(1*H*-imidazol-2-ylamino)propyl]-1*H*-indazol-5-yl}carbonyl)amino]-2-[(mesitylsulfonyl)amino]propanoic acid), SD-836 (Pharmaprojects), SD-7784 (Pharmaprojects), SD-7783 (Pharmaprojects), S-137 (N-({[1-(4-{[amino(imino)methyl]amino}butyl)vinyl]amino}acetyl)-3-pyridin-3-yl-beta-alanine), S-787 (Seattle et al.; 21st Ann. Meet. Amer. Soc. Bone Mineral Res., 30.9.-4.10.1999; SU 410), S 448 (N-{[(3-{[amino(imino)methyl]amino}benzoyl)amino]acetyl}-3-phenyl-β-alanine), SC 68448 (N-{[(3-{[amino(imino)methyl]amino}benzoyl)amino]acetyl}-3-(3,5-dichlorophenyl)-β-alanine), SC 56631 (N-{[(5-{[amino(imino)methyl]amino}pentanoyl)amino]acetyl}-3-pyridin-3yl-β-alanine), SC 69000 (4-[(3-{[amino(imino)methyl]amino}benzoyl)amino]-*N*-(isobutoxycarbonyl)phenylalanine), SC-65811 (*N*-{[(3-{[(benzylamino)carbonyl]amino}benzoyl)amino]acetyl}-3-pyridin-3-yl-b-alanine), SB 223245 (((2*S*)-7-{[(1*H*-benzimidazol-2-ylmethyl)(methyl)amino]carbonyl}-4-methyl-3-oxo-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepin-2-yl)acetic acid), SB 265123 ([(10S)-3-[3-(pyridin-2yl-amino)propoxy]-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-10yl]acetic acid), SB 267268 ([(4*S*)-3-oxo-8-[3-(pyridin-2-ylamino)propoxy]-2-(2,2,2-trifluoroethyl)-2,3,4,5-tetrahydro-1*H*-2-benzaz -pin-4-yl]acetic acid), SB 273005 (Lark et al.; 21st Ann. Me t. Amer. Soc. Bone Mineral Res., 30.9.-4.10.1999; SU201), CP-4632

((2*S*)-3-[(3-fluoro-4-[4-(1,4,5,6-tetrahydropyrimidin-2ylamino)piperidin-1yl]benzoyl)amino]-2-[(phenylsulfonyl)amino]propanoic acid), (2*S*)-3-({3-chloro-4-[4-(1,4,5,6-tetrahydropyrimidin-2-yl)piperidin-1-yl]benzoyl}amino)-2-[(phenylsulfonyl)amino]propanoic acid), SH306 (2S)-2-[(mesitylsulfonyl)amino]-3-[({1-[3-(pyridin-2-ylamino)propyl]-1H-indazol-5-yl}carbonyl)amino]propanoic acid, SB 273005 (Lark et al.; 21st Ann. Meet. Amer. Soc. Bone Mineral Res., 30.9.-4.10.1999; SU201) [(4S)-8-{2-[6-(Methylamino)pyridin-2-yl]ethoxy}-3-oxo-2-(2,2,2-trifluoroethyl)-2,3,4,5-tetrahydro-1H-2-benzazepin-4-yl]acetic acid, SC 72115 (3-(5-bromo-3-chloro-2-hydroxyphenyl)-N-({[3-(4,5-dihydro-1H-imidazol-2-ylamino)benzoyl]amino}acetyl)-beta-alanine).

Preferred are non-peptidic antagonists, particularly those which are orally available and $\alpha_v\beta_3$ integrin receptor antagonists selected from the group:
LM 609 (vitaxin), EMD 121974 (cyclo[R-G-D-f-(NMe)V]), L-7418415 ((2*S*)-2-[(phenylsulfonyl)amino]-3-({4-[2-(1,4,5,6-tetrahydropyrimidin-2-ylamino)ethoxy]benzoyl}amino)propanoic acid), SB 265123 ([(10S)-3-[3-(pyridin-2yl-amino)propoxy]-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-10yl]acetic acid), SB 267268 ([(4*S*)-3-oxo-8-[3-(pyridin-2-ylamino)propoxy]-2-(2,2,2-trifluoroethyl)-2,3,4,5-tetrahydro-1*H*-2-benzazepin-4-yl]acetic acid), SB 273005 (Lark et al.; 21st Ann. Meet. Amer. Soc. Bone Mineral Res., 30.9.-4.10.1999; SU201), SC 68448 (N-{[(3-{[amino(imino)methyl]amino}benzoyl)amino]acetyl}-3-(3,5-dichlorophenyl)-β-alanine), SC 69000 (4-[(3-{[amino(imino)methyl]amino}benzoyl)amino]-*N*-(isobutoxycarbonyl)phenylalanine and SC-65811 (*N*-{[(3-{[(benzylamino)carbonyl]amino}benzoyl)amino]acetyl}-3-pyridin-3-yl-b-alanine).
All mentioned compounds can also be applied as prodrugs. Prodrugs are substances which metabolise in vivo to the active compound. Examples for such metabolism are first pass metabolism (e.g. esters to free acids or carboxylates).

All mentioned compounds may be administered as such or in the form of their salts with physiologically tolerated acids or bases.

Preferred combinations of an endothelin blocker with an $\alpha_v\beta_3$ integrin receptor antagonist are selected from the preferred endothelin blockers and the preferred $\alpha_v\beta_3$ integrin receptor antagonists.

In a further preferred embodiment, orally available ET antagonists are combined with orally available $\alpha_v\beta_3$ integrin receptor antagonists.

"Orally available" means at least 10%, preferred 20% and more preferred 30% for ET antagonists and 30%, preferred 50% and more preferred 70% for $\alpha_v\beta_3$ integrin receptor antagonist. The oral availability is for the purpose of this invention defined as measured in dogs as described in WO 9806740.

The endothelin blocker in combination with the $\alpha_v\beta_3$ integrin receptor antagonist may be adminstered together in a pharmaceutical composition or simultaneous via separate ways or separate or temporal graduated.

Therefore, the invention further relates to a pharmaceutical composition, comprising an endothelin blocker and an $\alpha_v\beta_3$ integrin receptor antagonist.

This composition can be used as a medicament, particularly for curing or preventing cardiovascular disorders, such as atherosclerosis, restenosis after vessel injury or revascularisation treatment, angioplasty (neointima formation, smooth muscle cell migration and proliferation), myokard infarkt or heart failure.

In a preferred embodiment, the composition is used for the treatment or prevention of restenosis after vessel injury or revascularisation treatment.

The compounds of the invention can be administered orally or parenterally in a conventionally way (subcutaneously, intravenousely, intramusculary, intraperitoneally, rectally). Administration can also take place with vapours or sprays through the nasopharyngeal space. Oral administration is preferred.

The dosage depends on age, condition and weight of the patient and on the mode of administration. The two compounds can be formulated in a single pharmaceutical form or in separate pharmaceutical forms. Administration can be given in several single doses (.g. 2 to 4) or once or twice a day as depot form.

The weight ratio of $\alpha_v\beta_3$ integrin receptor antagonist to endothelin blocker conveniently is in the range of 1:100 to 100:1 preferably 1:10 to 10:1.

Advantageously, the dosage to be administered by means of a combination per day and kg amounts to 0,05 to 20 mg of an $\alpha_v\beta_3$ integrin receptor antagonist and 0,1 to 50 mg of an endothelin blok-

ker. In general, the total amount of an $\alpha_v\beta_3$ integrin receptor antagonist and an endothelin blocker to be administered daily amounts per kg to a maximum of 50 mg. When a hydrate or a pharmaceutically usable salt is used, then the above values are to be appropriately adjusted.

The compounds can be used individually or together in conventional solid or liquid pharmaceutical forms, e.g. as uncoated or (film-)coated tablets, capsules, powders, granules, suppositories, solutions, ointments, creams or sprays. These are produced in a conventional way. In these, the active substances can be processed with conventional pharmaceutical aids such as tablet binders, fillers, preservatives, tablet disintegrants, flow regulators, plasticizers, wetting agents, dispersants, emulsifiers, solvents, release slowing agents, antioxidants and/ or propellant gases (cf. H. Sucker et al. Pharmaceutische Technologie, Thieme Verlag, Stuttgart, 1978). The administration form obtained in this way normally comprises the active substance in an amount of from 0.1% to 99% by weight.

Treatment of a patient with a cardiovascular disease by a combination, composition and method according to the present invention may include concomitant use of further adjunctive agents, such as antiplatelet agents, e.g., aspirin, and anticoagulant agents, .g., heparin or low molecular weight heparin, or other drugs, e.g., b—blockers, angiotensin converting enzyme inhibitors, agents against reperfusion injury and others.

Subject of the present invention are also pharmaceutical compositions, comprising $\alpha_v\beta_3$ integrin receptor antagonist in an appr - priate container and an endothelin blocker in a separate container to be used according to the above—mentioned administration regiments.

Pharmaceutical packaging units prepared in accordance with the present invention may consist of an appropriate administration form comprising the $\alpha_v\beta_3$ integrin receptor antagonist, and an appropriate packaging unit comprising the endothelin blocker. The two active compounds are preferrably present in the packaging unit in two different containers, e.g. tablets. However, depending on the type of active compound, it may also be possible to provide both compounds in a single dosage form. Further, the pharmaceutical packaging units comprise instructions, for example in the form of a package leaflet prescribed for medicaments from which it follows that the administration of a therapeutically active amount of the $\alpha_v\beta_3$ integrin r ceptor antagonist advanta-

geously takes place in combination with administration of an endothelin blocker.

If applied separately, the administration of the endothelin blocker takes places before, simultaneously or after the administration of the $\alpha_v\beta_3$ integrin receptor antagonist.

Information regarding the manner of use can either be given in the information leaflet or as a packing overprint on the medical preparation which can be bought together with medicinal preparations which comprise $\alpha_v\beta_3$ integrin receptor antagonists. On the one hand, pharmaceutical packaging units comprising only appropriate administration forms of the $\alpha_v\beta_3$ integrin receptor antagonists can comprise such information e.g. in the form of package leaflets, wherein the combined administration together with endothelin blockers according to the present invention is mentioned. On the other hand, pharmaceutical packaging units comprising only endothelin blockers can comprise such information wherein the combined administration together with $\alpha_v\beta_3$ integrin receptor antagonists and the use according to the present invention is mentioned. A third alternative would be to provide pharmaceutical pakkaging units comprising an $\alpha_v\beta_3$ integrin receptor antagonist, endothelin blocker and an appropriate information about the combined use of both, e.g. the usual package leaflet.

Therefore, the invention further relates to a pharmaceutical trade package, comprising as pharmaceutical agent an endothelin blocker or/and an $\alpha_v\beta_3$ integrin receptor antagonist together with an instruction for use of this pharmaceutical agents in combination for simultaneous, separate, or temporal graduated application for the treatment or prevention of diseases.

Appropriate directions of use of the above-mentioned pharmaceutical agents are essential for commercialization of such pharmaceutical packages, comprising either the $\alpha_v\beta_3$ integrin receptor antagonist, endothelin blocker or a combination thereof.

Commercialization of appropriate pharmaceuticals by pharmaceutical companies is only possible when prior approval of such pharmaceutical agents and the respective administration regimens is achieved by the respective national Health Authorities, such as the FDA in the US or the CPMP Authority in Europe.

This includes but is not limited to performing clinical trials according to well-established procedures under the supervision of said pharmaceutical company which lateron intends to commercialize such pharmaceutical agents. This also includes filing of ap-

propriate documentation about the results of such clinical trials with the respective Health Authority in order to get marketing approval. The approval is in many cases restricted to certain administration protocols or regimens which have to be included in printed form in the accompanying information leaflet prescribed for medicaments.

Examples

Example 1

Integrin $\alpha_v\beta_3$ (human)/ Vitronectin (human) ELISA:

96-well plates (Costar, cat # 3369) were coated overnight at 4°C with 100 µl/well integrin $\alpha_v\beta_3$ (5 µg/ml) from human placenta in 50 mmol/l $NaHCO_3$ (pH 9.2). After (3x) washing with 0.05% Tween 20 in PBS, 50 µl of test buffer (0.1 % skimmed milk powder in 50 mmol/l Tris, 1 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, 10 µmol/l $MnCl_2$, 100 mmol/l NaCl ,0.2% Tween 20) were pipetted into each well followed by 1 µl DMSO (control) or 1 µl $\alpha_v\beta_3$ integrin receptor antagonist solution (1 mmol/l to obtain a final test concentration of 10 µmol/l) and by 50 µl vitronectin solution (2 µg/ml from human plasma. The wells were incubated for 2 h at room temperature and then washed again three times with 0.05% Tween 20 in PBS. Bound vitronectin was detected by incubation with 100 µl of peroxidase-coupled anti-vitronectin antibodies (0.5 µg/ml) in buffer containing 0.2 % Tween 20 and 0.1% milk powder for 2 h at room temperature. After three washing steps with 0.05% Tween 20 in PBS, TMB solution 100 µl/well was added and incubated for 40 seconds at 37 °C. The reaction was stopped by addition of 100 µl/well 2N H_2SO_4. Finally the absorbance as a measure for bound vitronectin was measured in a microplate photometer at 450 nm.

Example 2

Cellular Adhesion Assay (ELISA technique):

24-well plates were coated overnight at 4°C with human vitronectin 50 ng/well in 50 mmol/l $NaHCO_3$ pH 9.2. After washing with CHO-S-SFMII medium the wells were incubated for 1 h at 37 °C with recombinant CHO $\alpha_v\beta_3$ expressing cells (subtype avb3-A: clone X, subtype $\alpha_v\beta_3$-B: clone 5, subtype avb3-C: clone 18) in CHO-S-SFMII medium (Gibco 12052-015) at a concentration of $1 \cdot 10^6$ cells/ml with $\alpha_v\beta_3$ integrin receptor antagonist added to obtain final concentrations between 0.1 nmol/l and 10 µmol/l for 2-3 h at 37 °C. Plates then again were washed four times with CHO-S-SFMII medium. XTT (Roche 1465015) 500 µl/well in CHO-S-SFMII medium was added and incubated for 2-5 h at 37 °C. Finally the absorbance as a measure for the count of adhesive cells was measur d in a photometer at 450 nm.

Example 3
HASMC Migration Assay

The migration of the primary human aortic SMC's was performed using 24-well Transwell cell culture chambers with an 8 µm pore size polycarbonate membrane (Costar3422). The lower surface of the filter was coated with vitronectin or osteopontin by incubation with 600 µl DMEM / 0.2% albumin / ± integrin $\alpha_v\beta_3$-ligand (10 µg/ml); 3 hrs 37°C/5% CO_2. HASMC (Cascade Biologics; C-007-5C) were suspended in DMEM and 100 µl were placed in the upper compartment of the chamber (250-300000 cells/well) with or without $\alpha_v\beta_3$ integrin receptor antagonist at various concentrations. The incubation was carried out at 37°C/5% CO_2 for 24 hours. The non-migrated cells on the upper surface of the filter were removed by washing with PBS. The filters were transferred to a new 24-well plate and the lower compartments were incubated with 400 µl DMEM and 200 µl XTT (Roche 1465015) at 37°C/5% CO_2. After 24 hours the absorbance of 100 µl was measured at 450 nm.

Example 4
huCASMC Proliferation Assay

Cryopreserved primary human coronary artery smooth muscle cells (huCASMCs) were purchased from Clonetics® Cell Discovery Systems (catalog no. CC-2583). Cells were subcultured in T-75 flasks using smooth muscle cell growth medium-2 (SmGM-2) supplemented with 0.5 ng/ml recombinant human epidermal growth factor (hEGF), 5 % foetal bovine serum, 5 µg/ml insulin, 50 µg/ml Gentamicin / 50 ng/ml Amphotericin-B and human fibroblast growth factor-B (hFGF-B) 2 ng/ml (Clonetics® Cell cat. no. CC-3182). For the proliferation assay cells between passage 5-8 were seeded at 5000 cells/well in 96 well plates in a volume of 0.1 ml and allowed to attach for 24 hours. Thereafter proliferating cells were incubated with an endothelin blocker and/or an $\alpha_v\beta_3$ integrin receptor antagonist each at a concentration of 10^{-7}M for further 24 hrs. Cell proliferation was quantitated in quadruplicates 24 hrs later using a colorimetric cell proliferation ELISA (BrdU, Roche Diagnostics, cat. no. 1 647 229).
In vivo study

Example 5
Pig Model of Coronary Artery Restenosis

The pig coronary restenosis models is acknowledged as the only preclinical animal model with predictive value for the human pathology of restenosis. The findings show that the combination of ET receptor antagonists and $\alpha_v\beta_3$ integrin receptor antagonists re-

present a more effective means of preventing restenosis than treatment with either drug alone. In fact, given the higher predictive value of the pig restenosis model, the in vitro results suggest effective prevention of human restenosis with combinations of ET receptor antagonists and $\alpha_v\beta_3$ integrin receptor antagonists only, rather than monotherapy.

The study was performed in land race pigs applying PTCA only or PTCA plus implantation of a stent in the LAD according to standard clinical protocols.5 minutes prior to balloon inflation an ET_A receptor antagonist an $\alpha_v\beta_3$ integrin receptor antagonist or a combination thereof was administered intravenously and after recovery from anaesthesia the animals received the compound/s orally, subcutaneously or by continuous infusion for 4 or 12 weeks. At the end of the experiments the LAD was excised and frozen to allow receptor distribution studies and histological examination with assessment of intimal hyperplasia after angioplasty by determination of residual lumen and the neointima/media ratio.

Detailed in vivo Method:

Animals: Species: domestic pig
Sex: male and female
Age: 8 to 9 weeks
Weight: 20 to 30 kg

Breeder: Dortans, Schatthausen, Germany. The health status of the animals used is controlled by a veterinary surgeon.Before start of the experiment, animals are acclimatized for at least 1 week. During this period the animals are trained to receive a small amount of food (mashed potatoes) before they are fed their standard maintenance diet (Ssniff Spezialdiäten GmbH, Soest), so that the drug containing mixture is quantitatively eaten by each animal within a few minutes. Drinking water is available ad libitum.

Study design:

40 pigs of both sexes are allocated at random to one of the following treatment groups:

1. control
2. $\alpha_v\beta_3$ integrin receptor antagonist: in a range between 0.05 and 5 mg/kg/d s.c. or as an iv infusion between 0,01 and 1 mg/kg/h.
3. ET_A receptor antagonist in a range between 0.1 and 50 mg/kg/d p.o.

4. ET_A receptor antagonist plus integrin $\alpha_v\beta_3$ antagonist [in a ratio between 1 : 10 and 10 : 1 and a total dosis range between 0.1 and 50 mg/kg/d p.o. or 5 mg/kg/d s.c. or 0,01 to 1 mg/kg/h infusion]

administration: once daily between 07:00 and 9:00 a.m. subcutaneously or orally, by giving freshly prepared mashed potatoes mixed with a calculated amount of drug.

Duration of treatment: 4 or 12 weeks, beginning one day before angioplasty.

All animals will receive an i.v. bolus injection of a tenth of the oral dose just before the angioplasty

Experimental procedure : 24 h before surgical intervention, the animals are given 650 mg acetylsalicylic acid (ASS, Ratiopharm) and 30 mg nifedipine orally in addition to the test substance or placebo. For introduction and maintenance of general anaesthesia during angioplasty, the animals are given 2 mg/kg stresnil® i.m. (Azaperon), followed by 4 mg/kg metomidat® i.v. (Hypnodil, Janssen). Animals are intubated (Rüsch Mikrolaryngealtubus, I.D.: 4.0 mm) and ventilated with 75% N_2O and 25 % O_2 . An 8F sheath (Cordis, FI 33102-5700) is placed retrogradely in the right carotid artery. Adequate anticoagulation is achieved by intraarterial bolus injection of 7000 IU of unfractionated heparin (Thrombophob®, Nordmark, Uetersen). A standard PTCA guide catheter (Judkin left, powerbase 8F, ACS; mandrin = softguide, soft type, ACS) is then advanced via the aortic root into the left coronary artery under X-ray guidance. The balloon catheter (RX Elipse 0.014, ACS) is positioned in the first third of the left coronary artery using a 0.014'' PTCA guide wire (Galeo, Biotronic, Berlin). After X-ray control of its position, the balloon catheter is expanded by inflating the balloon twice to 10 atm for 30 sec. After deflating the balloon and after withdrawing it, an additional angiogram is made to verify the lesion.

Specimen collection: 28 or 92 days after balloon angioplasty the animals are anaesthetized as described above. Thereafter the animals receive a relaxant (Impretil®, hexacarbocholinbromide, 0.03 mg/kg i.v.), the chest is opened and the heart removed. The dilated coronary artery segments including the adjacent noninjured segments are then carefully dissected from the epicardial surface, transferred into PBS and tissue tek is injected into the artery. After freezing the artery is sectioned transversely into 4 mm pieces. 4 sections from each segment are used for analysis

(determination of area media, intima and lumen)after staining with hematoxylin-eosin (HE).

The cross sectional area of each segment is determined with digital morphometry. Neointimal thickness is determined as difference of the residual lumen area from the total area within the internal elastica lamina, which is considered as the normal lumen area. An uninjured proximal and distal segment of 4 mm length each was used as a reference lumen.

Parameters measured: Incidence of acute events (acute myocardial infarction, death, cardiac arrhythmia, coronary artery spasm, arterial dissection, haematoma), neointimal-, lumen-, media-, and adventitial area and mean thickness to be determined in 4 mm intervals of the dilated area. Angiographic lumen diameter before and after balloon inflation and at the end of the 28 or 29 day treatment period.

Evaluation: Results are expressed as mean ± SEM or as median. Comparisons between groups are made using Dunnett's test for unpaired samples. Correlations between injury score and neointima formation are used to compare the effect of different treatments.

The use of the combination of an endothelin blocker and an $\alpha_v\beta_3$ integrin receptor antagonists achieves a reduction of restenosis significantly more pronounced then one of the two treatments alone at the given doses. The combination of an ET blocker and an $\alpha_v\beta_3$ integrin receptor antagonists in doses too low to be effective alone is effective as a high mono-therapy with either agent and has less potential for side-effects than one principle alone.

Claims

1. Use of an endothelin blocker in combination with an $\alpha_v\beta_3$ integrin receptor antagonist for the manufacture of medicaments for the treatment or prevention of diseases.

2. Use as claimed in claim 1 for the treatment or prevention of cardiovascular disorders.

3. Use as claimed in claim 2 for the treatment or prevention of restenosis after vessel injury or revascularisation treatment.

4. Pharmaceutical composition, comprising an endothelin blocker and an $\alpha_v\beta_3$ integrin receptor antagonist.

5. Composition as claimed in claim 4 for use as a medicament.

6. Composition as claimed in claim 5 for curing cardiovascular disorders.

7. Use of a composition as claimed in claim 4 for the manufacture of a medicament for the treatment or prevention of diseases.

8. Use as claimed in claim 7 for the treatment or prevention of cardiovascular disorders.

9. Use as claimed in claim 8 for the treatment or prevention of restenosis after vessel injury or revascularisation treatment.

10. Trade package, comprising as pharmaceutical agent an endothelin blocker or/and an $\alpha_v\beta_3$ integrin receptor antagonist together with an instruction for use of this pharmaceutical agents in combination for simultaneous, separate, or temporal graduated application for the treatment or prevention of diseases.

Inhibtors of the Endothelin Signalling Pathway and $\alpha_v\beta_3$ Integrin receptor antagonists for Combination Therapy

Abstract

The invention relates to the use of an endothelin blocker in combination with an $\alpha_v\beta_3$ integrin receptor antagonist for the treatment or prevention of diseases, particularly to the use of a pharmaceutical composition, comprising an endothelin blocker and an $\alpha_v\beta_3$ integrin receptor antagonist, for the treatment or prevention of cardiovascular disorders, particularly for the treatment or prevention of restenosis after vessel injury or revascularisation treatment and to the pharmaceutical composition itself.

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(71) Applicant *(for all designated States except US)*: **BASF AKTIENGESELLSCHAFT** [DE/DE]; 67056 Ludwigshafen (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **AMBERG, Wilhelm** [DE/DE]; Schälzigweg 79, 68723 Schwetzingen (DE). **KLING, Andreas** [DE/DE]; Riegeler Weg 14, 68239 Mannheim (DE). **HORNBERGER, Wilfried** [DE/DE]; Goldener Winkel 14, 67434 Neustadt (DE).

(74) Common Representative: **BASF AKTIENGESELLSCHAFT**; 67056 Ludwigshafen (DE).

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(57) Abstract: The invention relates to the use of an endothelin blocker in combination with an $\alpha_v\beta_3$ integrin receptor antagonist for the treatment or prevention of diseases, particularly to the use of a pharmaceutical composition, comprising an endothelin blocker and an $\alpha_v\beta_3$ integrin receptor antagonist, for the treatment or prevention of cardiovascular disorders, particularly for the treatment or prevention of restenosis after vessel injury or revascularisation treatment and to the pharmaceutical composition itself.

INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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